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(54) Title: TREATMENT AND PREVENTION OF PROSTATIC DISEASE			
(57) Abstract			
<p>The present invention is related to a method for treating and preventing benign prostatic hyperplasia (BPH) and prostatic carcinoma by administering a therapeutically effective amount of a compound which binds to SHBG and antagonizes the SHBG-mediated effects of both estradiol and 5α-androstan-3α,17β diol by preventing the binding of estradiol and 5α-androstan-3α,17β diol. The present invention further relates to the compounds which bind SHBG and prevent the binding of estradiol and 5α-androstan-3α,17β diol as well as to a method of finding compounds which bind to SHBG and prevent the binding of estradiol</p>			

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TITLE OF THE INVENTION

TREATMENT AND PREVENTION OF PROSTATIC DISEASE

BACKGROUND OF THE INVENTION

5 Benign prostatic hyperplasia (BPH) and prostatic carcinoma are among the most common afflictions of aging men.

10 Benign prostatic hyperplasia is often treated surgically with a procedure known as transurethral resection of the prostate (TURP). Other surgical procedures performed to release the obstruction of urine include incision or stents. Castration has also resulted in regression of prostatic enlargement. Drug therapy for BPH has included alpha-1 15 blockers which treat the symptoms of the disease by alleviating obstructive symptoms, but do not effect the underlying cause of the disease, the enlarged prostate gland. Representative alpha-1 blockers used in the treatment of BPH include: prazosin, terazosin, doxazosin, tamsulosin and alfuzosin. These drugs relax prostatic smooth muscle tone, decreasing intraurethral pressure without affecting bladder pressure. Common side effects of these agents are dizziness, headache and fatigue.

20 Finasteride (17 β -(N-tert-butylcarbamoyl)-4-aza-5 α -androst-1-ene-3-one), which is marketed by Merck & Co., Inc., under the tradename PROSCAR \circledR , is an inhibitor of testosterone 5 α -reductase currently marketed for the treatment of benign prostatic hyperplasia. A 25 principal mediator of androgenic activity in the prostate is 5 α -dihydrotestosterone ("DHT"), formed locally in the prostate by the action of testosterone-5 α -reductase. Inhibitors of testosterone-5 α -reductase inhibit the conversion of testosterone (T) to DHT and serve to prevent or lessen symptoms of hyperandrogenic stimulation in the prostate. See especially United States Patent No. 4,377,584 assigned to Merck & Co., Inc., issued March 22, 1983. Finasteride's utility in the treatment of 30 prostatic carcinoma is also disclosed in the following documents: EP 0 285,382, published 5 October 1988; EP 0 285 383, published 5 October 1988; Canadian Patent no. 1,302,277; and Canadian Patent no. 1,302,276.

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Both prostatic carcinoma and BPH have been treated with antiandrogens. Nonsteroidal antiandrogens such as flutamide and casodex compete with DHT for cytosolic androgen receptor sites in the prostate cells. These non-steroidal antiandrogens do not substantially

5 change sexual potency and libido as the gonadotrophin releasing hormone agonists and progestogens do; however, these nonsteroidal antiandrogens often exhibit the undesirable tendency to feminize the male host (gynaecomastia) or initiate feed-back effects which would cause hyperstimulation of the testes.

10 Gonadotrophin-releasing hormone (GnRH) agonists such as nafarelin, buserelin, goserelin and leuprorelin all reduce the release of leutinizing hormone (LH) by desensitizing the GnRH receptors in the anterior pituitary gland. GnRH agonists are able to reduce the production of testosterone, induce shrinkage of prostate volume and reduce the

15 severity of urinary symptoms of BPH. Unfortunately, these drugs have adverse effects such as impotence and flushing, which discourage a majority of patients from continuing with the drugs. These androgen-suppressing agents are thus of inconsequential significance in BPH treatment, but are of major importance in the treatment of patients with

20 advanced prostatic cancer.

25 Progestogens, such as megestrol acetate, hydroxyprogesterone and medrogestone depress testosterone by inhibiting LH release and blocking androgen receptors, causing a reduction in prostatic volume. Adverse affects such as decreased libido and impotence have kept progestogens from common use in BPH treatment.

30 Thus, there still remains a need for additional therapies for BPH and prostatic carcinoma for individuals who cannot tolerate the side effects and/or do not experience adequate relief from presently available therapies.

Sex hormone binding globulin (SHBG) is a binding protein found in the serum of humans and dogs, but not rats, that binds testosterone, dihydrotestosterone and estradiol with high affinity (Kd at 37°C: DHT 1=nM; T =3 nM). The binding of each of the sex steroids

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appears to be competitive. Until the present invention, the function of SHBG was not defined, but it was thought to play a role in regulating the amount of "free" steroid that diffuses into target cells to interact with steroid receptors. This function was thought to be accomplished simply

5 by the fact of binding and was not believed to depend on a specific interaction of SHBG with cells. In addition to binding steroids, SHBG itself binds to receptor sites on plasma membranes.

SHBG appears to be a cellular regulator whose interactions with cells and ultimate effects thereon are controlled in two steps. STEP

10 1: Only unliganded SHBG binds to receptor. Inhibition of binding is related only to occupancy of its steroid-binding site, not to the nature of the steroid that is bound. Thus, the concept of free SHBG as an important biological entity must be considered. In normal men, about 40% of SHBG is unliganded; of that which is bound, testosterone accounts for

15 75% of the occupancy. Dunn et al., J. Clin. Endocrinol. Metab. 53:69-75 (1981). In women, over 80% of SHBG is unliganded. No single steroid accounts for as great a fraction of the occupancy as in men, but over half of the occupancy is accounted for by testosterone,

20 dehydroepiandrosterone, and $\Delta 5$ -androstenediol. It is evident that the nature of the steroids (e.g., their concentration and affinity for SHBG) that circulate lends some specificity to what is a relatively nonspecific system, if only the physical chemistry is considered. STEP 2: After unliganded SHBG binds to its receptor, a steroid may bind to the SHBG-

25 receptor complex, and, if it has biological activity, lead to the accumulation of intracellular cAMP and/or other post-binding events such as the translocation of the bound steroid.

Estradiol increases the amount of SHBG made by the liver. In men, both SHBG and the ratio of estradiol to testosterone in serum increase as a function of age.

30 William Rosner et al. (Nakhla et al., J. Clin. Endocrin. & Metab. 71(2): 498-404 (1990)) demonstrated that unliganded SHBG binds to human prostate cells and that the subsequent binding of estradiol causes a dose-dependent increase in cAMP. Once SHBG is bound to its receptor, it can then bind a steroid, resulting in dissociation of SHBG

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from its receptor (the half-time of this dissociation is about 30 hours.) Although this activity was first recorded in human prostate cancer cells (LNCaP cells), the effect was much more pronounced in primary cultures of BPH tissue. See Nakhla et al "Estradiol causes the rapid accumulation 5 of cAMP in human prostate" Proc Natl Acad Sci (USA) 91:5402-5405, 1994. A comparison of cultured fibroblastic and epithelial cells from BPH showed activity was confined to fibroblasts. The cAMP effect was 10 specific for estradiol. Although dihydrotestosterone (DHT) binds to SHBG, DHT had no effect on cAMP in BPH tissue. In the prostatic cancer cell line LNCaP, both DHT and estradiol activated the system, causing an increase in cAMP.

Furthermore, the antiestrogen tamoxifen did not block the effect of estradiol in increasing cAMP. This is presumably because tamoxifen does not compete for estradiol binding to SHBG.

15 Further, the potent estrogen diethylstilbestrol did not bind to SHBG and did not increase cAMP in prostate cells.

20 The steroid metabolite, 2-methoxyestradiol, has no known biological activity. However, 2-methoxyestradiol binds to SHBG more tightly than does estradiol. This binding does not produce the increase in cAMP observed with estradiol binding.

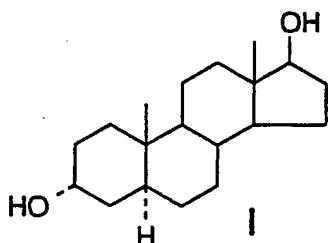
25 Androgens are widely acknowledged to be central to the pathogenesis of benign prostatic hyperplasia and prostatic carcinoma. However, these diseases of the prostate increase in prevalence as men age, a time when plasma androgens are falling. The decrease in total plasma androgens is amplified by an age-related increase in plasma SHBG that results in a relatively greater fall in free than in total androgens. In addition, estrogens have long been suspected to be important in BPH, but a direct effect on the human prostate has never been demonstrated. Estradiol, falling androgen levels, and rising SHBG 30 have been suggested to play a role in the pathogenesis of disease of the prostate. Estradiol acts in concert with SHBG to produce large increases in intracellular cAMP in human BPH and prostatic cancer tissue. As this increase is not blocked by an antiestrogen and not provoked by an

estrogen that does not bind to SHBG, it is not mediated by the classic intracellular estrogen receptor.

The present invention is related to a method for treating and preventing benign prostatic hyperplasia (BPH) and prostatic carcinoma by administering a therapeutically effective amount of a compound which binds to SHBG and antagonizes the SHBG-mediated effects of both estradiol and 5α -androstan- $3\alpha,17\beta$ diol by preventing the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ diol. The present invention further relates to a method of finding compounds which bind to SHBG and prevent the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ diol.

DETAILED DESCRIPTION OF THE INVENTION

5 α -Androstan-3 α ,17 β diol (3 α -diol) of structural formula I,



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is formed by the 3α reduction of dihydrotestosterone and/or the 17β reduction of androsterone. It is not thought to have intrinsic hormonal activity. Because it is an abundant metabolite of DHT in the skin, the plasma concentration of its glucuronide has been proposed as a marker of cutaneous androgen activity in hirsute women, although its usefulness in this regard is not certain. Similarly, prostatic 3α -diol is thought to simply reflect the disposition of DHT that eventuates in plasma 3α -diol glucuronide. 3α -diol has been implicated in prostatic disease because its administration leads to BPH in the dog. Because prostatic DHT in dogs given 3α -diol approximates that seen in spontaneous BPH, and because the administration of DHT also results in BPH in dogs, it is assumed but

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not proven that the mechanism whereby 3 α -diol participates in the genesis of BPH involves its oxidation to DHT.

As discussed in the background of the invention, during the last few years, evidence has been accumulated to support the hypothesis that steroid hormones exert effects through mechanisms in addition to those involving their classic intracellular receptors. One such mechanism is based on the observation that a number of cells have receptors on their plasma membranes for the plasma protein, SHBG. Also, discussed above is evidence that the SHBG-[sex hormone-binding globulin receptor] (RSHBG) complex is involved in a steroid activated signal transduction pathway that eventuates in the rapid generation of intracellular cAMP. In the prostates of patients with BPH, estradiol activates the SHBG-RSHBG complex and DHT competitively antagonizes this activation. In addition to estradiol, only one other steroid serves as an agonist in this system, 3 α -diol. Further, this system is present in canine prostate and is activated by the same two steroids.

The present experiments demonstrate that 3 α -diol, heretofore thought to be an inactive metabolite of DHT, is a hormone. It is an agonist in the SHBG-RSHBG system. The concentration causing a 1/2 maximal response is about equal to its concentration in the plasma of men. Further, only 3 α -diol and estradiol are agonists in this system; and both steroids induce BPH in dogs; all other endogenous steroids that bind to SHBG with high affinity antagonize the effects of the two agonists. Conversely, structurally related steroids that do not bind to SHBG, e.g. 3 α - and 3 β ,5 β -androstane diol, are neither agonists nor antagonists.

The estradiol engendered rise in cAMP is not mediated by the estrogen receptor. In BPH tissue, DHT is not an agonist, but antagonizes the effect of estradiol. DHT also antagonizes the effect of 3 α -diol. The same is true of testosterone. Thus, the two classic endogenous androgens that exert their effects by way of the intracellular androgen receptor serve as antagonists in the SHBG-RSHBG complex. All endogenous steroids that bind to SHBG are active as either agonists or antagonists.

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Although the human prostate contains an estrogen receptor, the role of estrogens in human BPH is unclear. It is certain, however, that estradiol synergises with 3α -diol in causing canine BPH. It is equally certain that these are the only two steroids that activate the RSHBG. It 5 had been previously asserted that the mechanism by which 3α -diol caused canine BPH was by its intraprostatic conversion to DHT. However, we show that 3α -diol may function as a direct effector of prostatic growth.

The instant invention involves a method of treating and/or 10 preventing BPH and prostatic carcinoma which comprises administering to a patient in need of such treatment a therapeutically effective amount of a compound which binds to SHBG and prevents the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ diol. In one embodiment of this 15 method, the compound which binds to SHBG and prevents the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ diol is administered in a dosage amount between 0.001 to 200.0 mg/day. In one class of this embodiment, the compound which binds to SHBG and prevents the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ diol is administered in a dosage amount of 20 from 0.01 to 50.0 mg/day, and in a sub-class of this embodiment, the compound which binds to SHBG and prevents the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ diol is administered in a dosage amount of about 0.1 to 5.0 mg/day. Compounds which bind to SHBG and prevent the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ diol can be determined by employing the assay described in Example 7.

25 In a second embodiment of this invention, the methods of treating and preventing benign prostatic hyperplasia and prostatic carcinoma comprise administration to a patient in need of such treatment of a compound which binds to SHBG and prevents the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ diol selected from: testosterone, 5β -androstan- $3\alpha,17\beta$ -diol, 5α -androstan- $3\beta,17\beta$ -diol, 5β -androstan-30- $3\beta,17\beta$ -diol, 2-methoxy-estradiol and Δ^5 -androstan- $3\alpha,17\beta$ -diol (also called androst-5-en- $3\alpha,17\beta$ -diol).

Preferred compounds that may be employed in the present invention include the following: 5α -androstan- $3\beta,17\beta$ -diol, 2-methoxy-

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estradiol and Δ^5 -androstan-3 α ,17 β -diol (also called androst-5-en-3 α ,17 β -diol).

The present invention has the objective of providing methods of treating and preventing diseases of the prostate including BPH and prostatic carcinoma by systemic, oral, parenteral or topical administration of a compound which binds to SHBG and prevents the binding of estradiol and 3 α -diol in a dosage amount between 0.001 to 200.0 mg/day, and more particularly, from about 0.01 to 50.0 mg/day, and most particularly 0.1 to 5.0 mg/day. The term "treating BPH" is intended to include alleviating the obstructive symptoms of BPH, and slowing and/or reversing the growth of the prostate. The term "preventing BPH" is intended to include preventing development of obstructive symptoms, and preventing the enlargement of the prostate. The term "treating prostatic carcinoma" is intended to include slowing and/or stopping the growth of prostatic carcinoma. The term "preventing prostatic carcinoma" is intended to include preventing the development of prostatic carcinoma in patients likely to develop prostatic carcinoma. Also, a compound which binds to SHBG and prevents the binding of estradiol and 3 α -diol may be co-administered with a 5 α -reductase 2 inhibitor, such as finasteride or epristeride; a 5 α -reductase 1 inhibitor such as 4,7 β -dimethyl-4-aza-5 α -cholestane-3-one, 3-oxo-4-aza-4,7 β -dimethyl-16 β -(4-chlorophenoxy)-5 α -androstane, and 3-oxo-4-aza-4,7 β -dimethyl-16 β -(phenoxy)-5 α -androstane as disclosed in WO 93/23420 and WO 95/11254; dual inhibitors of 5 α -reductase 1 and 5 α -reductase 2 such as 3-oxo-4-aza-17 β -(2,5-trifluoromethylphenyl-carbamoyl)-5 α -androstane as disclosed in WO 95/07927; nonsteroidal antiandrogens such as flutamide and casodex, and alpha-1 blockers such as prazosin, terazosin, doxazosin, tamsulosin, and alfuzosin.

The present invention also has the objective of providing suitable systemic, oral, parenteral and topical pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compositions containing as an active ingredient a compound which binds to SHBG and prevents the binding of estradiol and 5 α -androstan-3 α ,17 β diol can be administered in a wide variety of

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therapeutic dosage forms in conventional vehicles for systemic administration. For example, the compounds can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, 5 solutions, suspensions, syrups and emulsions. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. For oral administration, for example, the 10 compositions can be provided in the form of scored or unscored tablets containing 0.01, 0.05, 0.1, 0.2, 1.0, 2.0, 5.0, 10.0, 50.0 and 100.0 milligrams of the active ingredient for the adjustment of the dosage to the patient to be treated.

15 The compound which binds to SHBG and prevents the binding of estradiol and 3 α -diol may be administered in a pharmaceutical composition comprising the active compound in combination with a pharmaceutically acceptable carrier adapted for topical administration. Topical pharmaceutical compositions may be, e.g., in the form of a solution, cream, ointment, gel, lotion, shampoo or aerosol formulation 20 adapted for application to the skin. Topical pharmaceutical compositions useful in the method of treatment of the present invention may include about 0.001% to 0.1% of the active compound in admixture with a pharmaceutically acceptable carrier.

25 Advantageously, compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. The compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those 30 of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including

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type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound thereof employed. A physician of ordinary skill can readily determine 5 and prescribe the effective amount of the drug required to prevent, counter, arrest or reverse the progress of the condition. Optimal precision in achieving concentration of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the 10 distribution, equilibrium, and elimination of a drug.

In the methods of the present invention, the compound which binds to SHBG and prevents the binding of estradiol and 3 α -diol herein described in detail can form the active ingredient, and are typically administered in admixture with suitable pharmaceutical diluents, 15 excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

For instance, for oral administration in the form of a tablet or 20 capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Capsules containing the product of this invention can be prepared by mixing an active compound of the present invention with lactose and magnesium stearate, calcium stearate, starch, talc, or other 25 carriers, and placing the mixture in a gelatin capsule. Tablets may be prepared by mixing the active ingredient with conventional tabletting ingredients such as calcium phosphate, lactose, corn starch or magnesium stearate. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be 30 incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium

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stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like.

The liquid forms may be administered in suitably flavored
5 suspending or dispersing agents such as the synthetic and natural gums, for example, tragacanth, acacia, methyl-cellulose and the like. Other dispersing agents which may be employed include glycerin and the like. For parenteral administration, sterile suspensions and solutions are desired. Isotonic preparations which generally contain suitable
10 preservatives are employed when intravenous administration is desired.

Topical preparations containing the active drug component can be admixed with a variety of carrier materials well known in the art, such as, e.g., alcohols, aloe vera gel, allantoin, glycerine, vitamin A and E oils, mineral oil, PPG2 myristyl propionate, and the like, to form, e.g.,
15 alcoholic solutions, topical cleansers, cleansing creams, skin gels, skin lotions, and shampoos in cream or gel formulations. See, e.g., EP 0 285 382.

The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small
20 unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Compounds of the present invention may also be delivered by the use of monoclonal antibodies as individual carriers to which the
25 compound molecules are coupled. The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamidephenol, polyhydroxy-ethylaspartamidephenol, or polyethyleneoxidepolylysine substituted with
30 palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals,

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polydihydropyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

The present invention also provides for the use of a compound which binds to SHBG and antagonizes the SHBG-mediated effects of both estradiol and 5 α -androstan-3 α ,17 β diol by preventing the binding of estradiol and 5 α -androstan-3 α ,17 β diol in the preparation of a medicament useful in the treatment of diseases of the prostate.

The present invention also relates to a method for identifying compounds useful for the treatment and prevention of BPH and prostate cancer comprising:

- 10 (a) incubating samples of the compound to be tested at a selected concentration in diluted pregnancy serum, dog SHBG or human SHBG containing a labelled steroid selected from labelled dihydrotestosterone, labelled estradiol, and labelled testosterone;
- 15 (b) separating the SHBG-bound steroid from non-bound steroid to allow quantitation of the amount of steroid bound to SHBG;
- 20 (c) determining the amount of steroid bound to SHBG; and
- (d) determining the IC50 of the compound.

The labelled steroid may be radioactively labelled, fluorescently labelled, chemiluminescently labelled or otherwise labelled. Preferably, labelled steroid is radioactively labelled. Most preferably, the labelled steroid is tritiated dihydrotestosterone, and the amount of label is measured by measuring the amount of radioactivity present in the supernatant. It is preferred to repeat steps (a) through (c) at least 5 times, each at a different concentration of compound. Preferably, the pregnancy serum, dog SHBG, or human SHBG is diluted 1:20 in sodium phosphate buffer, pH 7.0, made 0.15 M in NaCl, and 0.5 mL of the diluted solution is aliquoted into test tubes and incubated with 0.1 nM tritiated DHT. When employing dog or human SHBG, it is desirable to start with purified dog or human SHBG. It is preferred that the concentration of compound to be

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tested is within a range of 0.001 to 100 nM, and that the incubation occur at room temperature for about 15 minutes and then in an ice bath for an additional 15 minutes. Preferably, the protein is precipitated by the addition of 0.5 mL saturated ammonium sulphate solution. Most 5 preferably, following addition of the ammonium sulphate, the diluted incubated mixture is shaken, preferably on a vortex mixer, to avoid locally high concentrations of salt. Preferably, to further precipitate the proteins, the tubes are centrifuged most preferably for 10 min at 8000 rpm. Then a measured sample of the supernatant is removed, preferably 10 0.5 mL, and the amount of label is measured.

Preferably, the IC₅₀ of the compound is determined by plotting the amount of radioactive DHT as a function of the logarithm of the concentration of the compound. It is preferred to determine the amount of radioactive DHT by subtracting the radioactivity of the 15 supernatant from the total radioactivity added to tube.

Preferably, those compounds with an IC₅₀ < 10 nM are reassayed in the human BPH tissue cAMP assay to determine whether the compound is an agonist or antagonist of estradiol and 3 α -diol. Generally, this assay comprises the steps:

- 20 (a) adding a sufficient amount unliganded SHBG to minces of prostatic tissue in culture to saturate their SHBG receptors;
- (b) removing excess SHBG, preferably by washing;
- (c) adding a known concentration of the compound to be 25 tested; and
- (d) determining intracellular cAMP.

If the compound is not an agonist (does not cause an increase in intracellular cAMP), then the following steps may be performed to confirm the antagonistic activity of the compound:

- 30 (e) adding a sufficient amount unliganded SHBG to minces of prostatic tissue in culture to saturate their SHBG receptors;
- (f) removing excess SHBG, preferably by washing;

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- (g) adding a compound selected from estradiol and 5α -androstan- $3\alpha,17\beta$ diol;
- (h) adding a known concentration of the compound to be tested; and
- 5 (i) determining intracellular cAMP.

Compounds of interest having an $IC_{50} < 10$ nM and found to be an antagonist in the human BPH tissue cAMP assay may be tested in dog models of BPH and subsequently in humans for treatment of the symptoms of BPH. In the dog model of BPH, castrate dogs are treated with estradiol and 5α -androstane diol in the presence and absence of the test compound. Prostate growth is monitored, preferably by MRI. Old dogs having BPH can also be treated with test compound to determine whether their enlarged prostate gland shrinks in response to the test compound. Again, prostate size is monitored, preferably by MRI.

The methods of treating and preventing diseases of the prostate including BPH and prostatic carcinoma described herein may be further illustrated by the following examples.

20

GENERAL METHODS

GENERAL METHOD 1

SHBG

SHBG, containing 1 mol DHT/mol SHBG, was isolated from pregnancy plasma by the modification (Khan et al., "Size isomers of testosterone-estradiol-binding globulin exist in the plasma of individual men and women" Steroids 45:463-72 (1985)) of our original method as described in Rosner et al., "Isolation and characterization of the testosterone-estradiol-binding globulin from human plasma: Use of a novel affinity column," Biochemistry, 14:4813-20 (1985). Its purity was verified by polyacrylamide gel electrophoresis (PAGE), sodium dodecyl sulfate PAGE (SDS-PAGE), and immunoelectrophoresis against antiwhole human antiserum. Examination by all of these methods revealed an absence of contaminants. It was stored at -20 C in 50 mM

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Tris-HCl, 50 mM CaCl₂ (pH 7.4) and 10% glycerol (Tris-Ca buffer) and used within 6 months of isolation. I

5 Steroids were stripped from SHBG with dextra-coated charcoal (2.5%, wt/vol). In preliminary experiments, the completeness of the stripping procedure was ensured by stripping a solution of 8 μ M SHBG-DHT which had been equilibrated with 10⁶ cpm [³H]DHT. The recovery of SHBG was 85%.

10 The following procedure was used to replace DHT, in a solution of SHBG-DHT with another steroid. Five milliliters of SHBG-DHT (1.1 μ M) in Tris-Ca buffer were placed in an AMICON ultrafiltration cell (model 52) fitted with a YM-10 membrane and gently stirred, at room temperature, with a 5-fold molar excess of the steroid to be exchanged. After 30 minutes, the temperature was decreased to 4°C, and incubation was continued for an additional 30 min. At that time, 40 15 mL ice-cold Tris-Ca buffer were added and 5 min later the solution was concentrated to the original volume (5 mL). Addition of 40 mL ice-cold buffer and concentration were repeated three times, after which the solution was warmed to room temperature, and a fresh 5-fold molar excess of the replacement steroid was added again. The entire procedure 20 was repeated several times. The exchange was monitored, as appropriate, with [³H] testosterone or [³H] estradiol. On the basis of radioactivity bound to SHBG, exchange was more than 90% complete for testosterone after 3 exchanges, and for estradiol after four exchanges. 2-
25 [³H]methoxyestradiol is not commercially available. Since it binds to SHBG more tightly than either estradiol or testosterone, we assumed that four exchanges were more than adequate. To control for any damage to SHBG which might affect experimental outcomes, DHT itself was carried through four exchanges.

30 Canine SHBG (cSHBG) was isolated as described by Suzuki et al. ("Purification and characterization of testosterone-binding globulin of canine serum" J. Biochem. 85:1195-12203(1979)), and its purity validated as for human SHBG (hSHBG). It too was stripped of steroids before use.

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GENERAL METHOD 2

Prostatic Tissue Explants

Human prostatic tissue was obtained at the time of surgery for BPH. Canine prostatic tissue was obtained by open surgery at the 5 time of sacrifice (parenteral phenobarbital) of 2-3 year old purebred beagles. The tissue was divided into approximately 5 mm³ cubes and placed in 60 mm Primaria culture dishes (Becton Dickinson Labware) in RPMI-1640 (GIBCO Laboratories, Grand Island, NY) with 5% fetal bovine serum containing 100 units/mL penicillin, 100 µg/mL 10 streptomycin sulfate and 0.25 µg/mL amphotericin, for 2-3 days. It was minced into 1 mm³ portions and transferred to 16 mm wells in serum-free medium (0.5 mL RPMI-1640) for about 18 h before beginning an experiment. Additions, e.g., SHBG, steroids, controls, were made in serum-free RPMI-1640.

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GENERAL METHOD 3

Membrane-bound adenylate cyclase activity and accumulation of intracellular cAMP are determined as previously described in Nakhla et al. "Induction of adenylate cyclase in mammary 20 carcinoma cell line by human corticosteroid binding globulin" Biochem Biophys Res Commun. 153:1012-8 (1988).

Protein was measured by the method of in Bradford "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding" Anal. Biochem. 72: 25 248-53 (1976).

GENERAL METHOD 4

LNCaP cells

LNCaP cells (a human prostatic carcinoma cell line, passage 30 9) were obtained from the American Type Culture collection (Rockville MD). Cells were grown in monolayers in Corning culture flasks (Corning Laboratory Science Co., Corning, NY) in RPM I-1640 medium supplemented with L-glutamine (300 mg/L) 10% fetal bovine serum

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(Gibco, Grand Island, NY) and antibiotics (100 U/mL penicillin G NAA, 100 µg/mL streptomycin sulfate, and 0.25 µg/mL amphotericin B).

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EXAMPLE 1

Effects of different SHBG-steroid combinations on cAMP accumulation

LNCaP cells were placed in Dulbecco's Modified Eagle's serum-free medium. Cells, (1 mg protein/ mL) were incubated for 14 minutes at 37°C with 1 µM SHBG-steroid or buffer (control) and the 10 phosphodiesterase inhibitor isobutylmethylxanthine (0.1 mM). They were then centrifuged and extracted with trichloroacetic acid for measurement of cAMP as described previously.

Results

15 The four different SHBG-steroid complexes were evaluated at a single SHBG-steroid concentration (1 µM). The exposure of cells to SHBG-steroid complexes took place for 15 minutes. Both free steroid and unliganded SHBG exist in the equilibrium mixture of 1 µM SHBG-steroid. The calculated concentrations of free SHBG and free steroids for SHBG-DHT, SHBG-testosterone, and SHBG-estradiol were 31, 52, and 20 65 nM respectively. (The association constants used in the calculation were $1.0 \times 10^9 \text{ M}^{-1}$ (DHT), $3.5 \times 10^8 \text{ M}^{-1}$ (testosterone), and $2.2 \times 10^8 \text{ M}^{-1}$ (estradiol).) The $t_{1/2}$ for DHT's dissociation from SHBG is 1.7 min. and those for testosterone and estradiol are substantially less. Thus, there 25 was ample time for the various steroids to equilibrate between the two pools of SHBG (free and receptor bound) and for the steroid to activate receptor-bound SHBG and set the adenylate cyclase system in motion.

To control for damage to SHBG during the course of substituting other steroids for DHT in the initially isolated SHBG-DHT complex, the SHBG-DHT complex was taken through the exchange 30 procedure four times using DHT as the exchanging steroid. This procedure resulted in a 14% decrease in activity compared to that of SHBG-DHT which was used without exchange. However, the exchanged SHBG was still significantly more active in causing an increase in cAMP than any of the other SHBG-steroid complexes.

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The most telling part of this experiment, however, was the lack of any statistically significant difference between SHBG-2-methoxyestradiol and the buffer control. This steroid metabolite binds to SHBG more tightly than either testosterone or estradiol, but is without 5 activity as a steroid hormone. DHT, in the absence of SHBG, was not different from the buffer control.

EXAMPLE 2

Effect on cAMP of adding steroids to cells to which unliganded SHBG 10 was already bound

To test the hypothesis that stimulation of adenylate cyclase activity and cAMP proceeds in the sequence: binding of unliganded SHBG to its receptor, binding of a steroid to the SHBG-receptor 15 complex, adenylate cyclase activation, and, finally cAMP formation, the effect on cAMP of adding steroids to LNCaP cells to which unliganded SHBG was already bound was tested. Cells (0.6 to 1.6 mg protein/mL) were placed in serum-free medium (see Example 1) and then incubated for 1 h at 37°C with 50 nM unliganded SHBG. The cells were washed 20 once with serum-free medium and then incubated for 15 minutes in serum-free medium containing varying concentrations of steroid and isobutyl-methylxanthine. The percent increase in cAMP after adding 50 nM unliganded SHBG, compared to the addition of buffer was 7.8% \pm 3.8%. LNCaP cells whose receptors were occupied by unliganded 25 SHBG, but not those with unoccupied receptors, showed a dose-dependent increase in their content of cAMP subsequent to the addition of DHT or estradiol. At concentrations of DHT more than 50 nM, the response declined. As with the addition of the SHBG-2-methoxy-estradiol complex in Example 1, addition of 2-methoxyestradiol to cells 30 whose receptors were occupied by unliganded SHBG had no effect on cAMP accumulation. By comparison, the response to estradiol ultimately achieves as great a magnitude as that to DHT, but it requires about a 20-fold greater final concentration (1 μ M) to achieve this.

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EXAMPLE 3

The overall experimental design was to add sufficient unliganded SHBG to minces of prostatic tissue in culture to saturate their SHBG receptor. After washing to remove excess SHBG, appropriate 5 concentrations of DHT or estradiol were added, and 15 minutes later the experiments were terminated and intracellular cAMP was determined. In the absence of SHBG, neither DHT nor estradiol affected a change in cAMP. SHBG without steroid caused a small increase in cAMP compared to buffer (48%, p , 0.03, n=20). In contrast, in the presence of 10 receptor-bound SHBG, estradiol caused a robust, dose-dependent, increase in cAMP. Surprisingly, DHT, which binds to SHBG with a 4.5 fold greater affinity than estradiol, did not activate the SHBG-receptor adenylate cyclase system in prostatic tissue.

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EXAMPLE 4

Prostate explants from normal dogs and patients with BPH were cultured in serum-containing medium for 3 days with twice daily changes in medium. Before starting an experiment, they were placed in serum-free medium for 24 h. Highly purified dog or human SHBG was 20 added to the appropriate explants for 3 h and non-receptor bound SHBG removed with 2 washes. Various concentrations of appropriate steroids were added and intracellular cAMP was assayed 15 minutes later.

Under no circumstance did any steroid, in the absence of SHBG, or SHBG in the absence of steroids affect cAMP. In the dog, 25 both estradiol, and 5 α -androstane-3 α ,17 β -diol (3 α -diol) caused increases in cAMP. At 100 nM, both resulted in about a 65-fold increase compared to blanks. For each, a one-half maximum response was somewhat less vigorous but more sensitive. In humans, the response to 3 α -diol was maximal (11 fold) at 10 nM and half-maximal at 0.4 nM. The response to 30 100 nM estradiol was approximately equal to 10 nM 3 α -diol. Testosterone, DHT, 5 α -androstane-3 β ,17 β -diol, 5 β -androstane-3 α ,17 β -diol, and 5 β -androstane-3 β ,17 β -diol, and Δ 5-androstane-3 β ,17 β -diol were without effect.

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In those organs in which testosterone functions as a prohormone, the steroid that activates the androgen receptor is the testosterone 5 α -reduced metabolite, DHT. DHT is metabolized further to 3 α - and 3 β ,5 α -androstaneadiol in a reversible manner. 3 α -Diol is known 5 to produce BPH in the dog and to synergize with estradiol in this effect. The data in this example show a direct effect of 3 α -diol in dog and human prostate, and raise the possibility that previously observed effects of 3 α -diol *in vivo* may be mediated without conversion to DHT.

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EXAMPLE 5

Experimental Design: The overall experimental design was to add sufficient unliganded SHBG (50 nM) to minces of prostatic tissue 15 in culture to saturate their SHBG receptors. After washing to remove excess SHBG, appropriate concentrations of the indicated steroids were added in the specified sequences and, 20 minutes after the last addition, the experiments were terminated and cAMP was determined by commercial ELISA (Oxford Biomed. Research, Inc., Oxford MI) as 20 before (Nakhla et al "Estradiol causes the rapid accumulation of cAMP in human prostate" Proc Natl Acad Sci (USA) 91:5402-5405, 1994.) All incubates contained isobutyl-methylxanthine (0.1 mM).

Results: Both estradiol and 3 α -diol added to human 25 prostatic minces, whose receptors had been saturated with SHBG, caused a dose-dependent, robust increase in cAMP. The same steroids added in the absence of SHBG were without effect. The Table below demonstrates the effect of estradiol or 3 α -diol with SHBG on cAMP in prostatic minces. For the human, the data are the mean \pm SEM of experiments carried out on tissue obtained from three patients, each in triplicate. For the dog, the data are the mean \pm SEM of triplicate 30 experiments from tissue obtained from one of two dogs.

Table I:

	Control	SHBG	3 α -diol (μ M)			Estradiol (μ M)		
			1	10	100	1	10	100
Human	0.111 ± 0.021	0.138 ± 0.026	0.647 ± 0.089	1.183 ± 0.149	1.206 ± 0.104	0.423 ± 0.049	0.703 ± 0.068	1.075 ± 0.217
Dog	0.395 ± 0.052	0.428 ± 0.048	5.478 ± 0.673	10.045 ± 0.976	22.72 ± 3.037	5.236 ± 0.739	13.4 ± 1.494	22.943 ± 2.006

units = ng cAMP/mg protein

In humans, the half maximal response occurred at 1.2 nM
 5 3 α -diol and 5.0 nM estradiol. Similar results were obtained using canine
 prostates and cSHBG. However, in the dog, the maximum response was
 greater, about 60 fold rather than the 11 fold seen in human prostate.
 Further, in the canine prostate, estradiol and 3 α -diol are equipotent.

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EXAMPLE 6Agonist and antagonist activity of steroids for RSHBG

We examined a variety of steroids in addition to estradiol
 and 3 α -diol for their ability to either stimulate the RSHBG, or inhibit the
 stimulation caused by 3 α -diol. The steroids were chosen either because
 15 they are known to bind tightly to SHBG (testosterone, DHT, 2-
 methoxyestradiol, Δ 5-androstene-3 β ,17 β -diol, 5 α -androstan 3 β ,17 β -
 diol), or because they are structurally related to 3 α -diol but do not bind to
 SHBG (the two isomers of 5 β -androstaneadiol.) None of these steroids
 were agonists in this system. Experiments were done in triplicate twice
 20 and are presented as the quotient: Experimental/control ± SEM as shown
 in Table 2. For agonist activity, steroids (10 nM) or vehicle (control)
 were examined for their ability to generate cAMP in prostatic minces
 whose receptors had been saturated with SHBG. Note that the control in
 the agonist activity experiments was unliganded SHBG + vehicle. For
 25 antagonist activity, the RSHBG in prostatic minces was saturated with
 hSHBG, incubated for 15 min with vehicle or steroid (50 nM) and then

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exposed to 10 nM 3 α -diol for 20 min. Note that the control in this experiment is SHBG + vehicle + 3 α diol. The steroids tested are 2-methoxy-estradiol (2MeOE2); Δ^5 -androstane-3 β ,17 β -diol (Δ^5); 5 β -androstan3 α ,17 β -diol (3 α ,5 β); 5 β -androstan3 β ,17 β -diol (3 β ,5 β); 5 α -androstan-3 β ,7 β -diol (3 β ,5 α).

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The data are summarized in Table 2.

Table 2: Agonist and Antagonist Activity of steroids for RSHBG

Activity	cAMP (Experimental / Control)							
	3 α ,5 α	3 β ,5 α	3 α ,5 β	3 β ,5 β	Δ^5	DHT	T	2MeO-E2
Agonist	12.7 ± 4.59	1.30 ± 0.19	1.30 ± 0.09	1.08 ± 0.07	1.04 ± 0.11	1.48 ± 0.41	1.49 ± 0.32	0.779 ± 0.182
Ant-agonist	1.000 ± 0.014	0.323 ± 0.152	1.010 ± 0.086	0.852 ± 0.002	0.062 ± 0.090	0.341 ± 0.046	0.224 ± 0.046	0.163 ± 0.021

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None of the steroids were agonists in this system. On the other hand, all of the steroids that bind to SHBG antagonized the effect of 3 α -diol, while the two 5 β -androstanediols did not.

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EXAMPLE 7
Identification of antagonists of estradiol and androstanediol useful for the treatment and prevention of BPH and prostate cancer.

Compounds that bind to SHBG are either agonists or antagonists. To select compounds that bind with appropriately high affinity, pregnancy serum is first diluted approximately 1:20 in sodium

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phosphate buffer, pH 7.0, made 0.15 M in NaCl. Then 0.5 mL of the diluted serum is aliquoted into test tubes is incubated with 0.1 nM tritiated-dihydrotestosterone (approximately 45 - 100 Ci/mmol) in the absence and presence of varying concentration of binding competitors (0.001 - 100 nM) for 15 min at room temperature. The tubes are then transferred to an ice bath and incubated for a further 15 min. 0.5 mL of

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saturated ammonium sulphate solution is then added while the diluted serum is being shaken on a vortex mixer to avoid locally high concentrations of salt. The tubes are centrifuged for 10 min at 8000 rpm and then 0.5 mL of the supernatant is counted. The amount of radioactive 5 DHT in the precipitate (determined by subtraction) is plotted as a function of the logarithm of the concentration of the competitor molecule and an IC₅₀ determined. The lower the IC₅₀ the more potent the competitor for tritiated-DHT binding to SHBG. Those compounds competing with an IC₅₀ < 10 nM are reassayed using human BPH tissue 10 in cultures containing pure human SHBG to determine whether the compounds are agonists or antagonists of estradiol and androstanediol in the human BPH tissue cAMP assay (Nakhla et al., J. Clin. Endocrin. & Metab. 71(2): 498-404 (1990)). The compounds found to be antagonists may be further tested in dog models of BPH and subsequently in humans 15 for treatment of the symptoms of BPH.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the casual variations, adaptations, modifications, 20 deletions, or additions of procedures and protocols described herein, as come within the scope of the following claims and its equivalents.

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WHAT IS CLAIMED IS:

1. A method of treating or preventing diseases of the prostate comprising administering to a person in need of such treatment a therapeutically effective dose of a pharmaceutical composition comprising a compound which binds to SHBG and prevents the binding of estradiol and 5 α -androstan-3 α ,17 β diol.
5
2. The method of Claim 1 wherein the dosage amount of the compound is from 0.001 to 200 mg/day.
10
3. The method of Claim 1 wherein the dosage amount of the compound is from 0.01 to 50 mg/day.
15
4. The method of Claim 1 wherein the dosage amount of the compound is from 0.1 to 5 mg/day.
19
5. The method of Claim 1 wherein the disease of the prostate is benign prostatic hyperplasia.
20
6. The method of Claim 1 wherein the disease of the prostate is prostatic cancer.
25
7. The method of Claim 1 wherein the pharmaceutical composition is administered systemically.
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8. The method of Claim 1 wherein the pharmaceutical composition is administered orally.
35
9. The method of Claim 1 wherein the pharmaceutical composition is administered topically.
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10. The method of Claim 1 wherein the compound which binds to SHBG and prevents the binding of estradiol and 5 α -androstan-3 α ,17 β diol is selected from

5 testosterone,
5 α -androstan-3 β , 17 β -diol,
2-methoxy-estradiol, and
 Δ^5 -androstan-3 α , 17 β -diol.

10 11. The method of Claim 1 wherein the compound which binds to SHBG and prevents the binding of estradiol and 5 α -androstan-3 α ,17 β diol is selected from

15 5 α -androstan-3 β , 17 β -diol,
2-methoxy-estradiol, and
 Δ^5 -androstan-3 α , 17 β -diol.

12. The method of Claim 1 additionally comprising the administration of a compound selected from:

- (a) a 5 α -reductase 2 inhibitor;
- (b) a 5 α -reductase 1 inhibitor;
- (c) a dual inhibitor of 5 α -reductase 1 and 5 α -reductase 2;
- (d) a nonsteroidal antiandrogen;
- (e) an alpha-1 blocker.

25 13. The method of Claim 12 wherein:

(a) the 5α -reductase 2 inhibitor is selected from finasteride and epristeride;

(b) the 5α -reductase 1 inhibitor is selected from 4,7 β -dimethyl-4-aza- 5α -cholestan-3-one, 3-oxo-4-aza-4,7 β -dimethyl-16 β -(4-chlorophenoxy)- 5α -androstane, and 3-oxo-4-aza-4,7 β -dimethyl-16 β -(phenoxy)- 5α -androstane;

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5 (c) the dual inhibitor of 5 α -reductase 1 and 5 α -reductase 2 is 3-oxo-4-aza-17 β -(2,5-trifluoromethylphenylcarbamoyl)-5 α -androstane;

10 (d) the nonsteroidal antiandrogen is selected from flutamide and casodex; and

15 (e) the alpha-1 blocker is selected from prazosin, terazosin, doxazosin, tamsulosin, and alfuzosin.

20 14. A method for determining whether a compound binds to SHBG and prevents the binding of estradiol and 5 α -androstan-3 α ,17 β diol comprising:

25 (a) incubating samples of the compound to be tested at a selected concentration in diluted pregnancy serum, pure dog SHBG or pure human SHBG containing a labelled steroid selected from dihydrotestosterone, estradiol, and testosterone;

30 (b) separating the SHBG-bound steroid from non-bound steroid to allow quantitation of the amount of steroid bound to SHBG;

(c) determining the amount of steroid bound to SHBG; and

(d) determining the IC50 of the compound.

15. The method of Claim 14 wherein steps (a) through (c) are repeated at least five times, each time at a different concentration of compound.

30 16. The method of Claim 14 wherein the compound is tested at concentrations between 0.001 and 100 nM.

17. The method of Claim 14 wherein the labelled steroid is tritiated dihydrotestosterone, the SHBG-bound steroid is separated from non-bound steroid by precipitation, and the amount of steroid bound to SHBG is measured by removing a measured amount of the supernatant

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from the precipitated mixture and measuring the radioactivity in the measured amount of supernatant.

18. A method for finding a compound useful for treating
5 and preventing diseases of the prostate comprising:

- 10 (a) incubating samples of the compound to be tested at a selected concentration in diluted pregnancy serum, dog SHBG or human SHBG containing a labelled steroid selected from labelled dihydrotestosterone, labelled estradiol, and labelled testosterone;
- (b) separating the SHBG-bound steroid from non-bound steroid to allow quantitation of the amount of steroid bound to SHBG;
- 15 (c) determining the amount of steroid bound to SHBG;
- (d) determining the IC₅₀ of the compound; and
- (e) determining whether the compound is an agonist or antagonist.

19. The method of Claim 18 wherein the determination of
20 whether the compound is an antagonist or agonist comprises the following additional steps:

- 25 (f) adding a sufficient amount unliganded SHBG to minces of prostatic tissue in culture to saturate their SHBG receptors;
- (g) removing excess SHBG from the saturated minces of prostatic tissue;
- (h) adding a known concentration of the compound to be tested to the washed minces; and
- (i) determining intracellular cAMP.

30 20. The method of Claim 19 wherein the determination of whether the compound is an antagonist or agonist comprises the following additional steps:

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- (j) adding a sufficient amount unliganded SHBG to minces of prostatic tissue in culture to saturate their SHBG receptors;
- 5 (k) removing excess SHBG from the saturated minces of prostatic tissue;
- (l) adding a known concentration of the compound to be tested to the washed minces; and
- (m) determining intracellular cAMP.

10 21. A pharmaceutical composition comprising:
(a) a compound which binds to SHBG and prevents the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ -diol; and
(b) a pharmaceutically acceptable carrier.

15 22. The composition of Claim 21 additionally comprising a compound selected from:
(a) a 5α -reductase 2 inhibitor;
(b) a 5α -reductase 1 inhibitor;
20 (c) a dual inhibitor of 5α -reductase 1 and 5α -reductase 2;
(d) a nonsteroidal antiandrogen; and
(e) an alpha-1 blocker.

25 23. The use of a compound which binds to SHBG and prevents the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ -diol for the manufacture of a medicament useful for preventing diseases of the prostate.

30 24. The use of a compound which binds to SHBG and prevents the binding of estradiol and 5α -androstan- $3\alpha,17\beta$ -diol for the manufacture of a medicament useful for treating diseases of the prostate.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08873

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/56

US CL :514/715,729,738,739

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

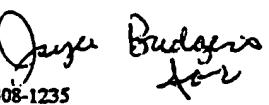
U.S. : 514/715,729,738,739

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,310,523 (NEUMANN) 12 January 1982, see the Abstract.	1-22

<input type="checkbox"/>	Further documents are listed in the continuation of Box C.	<input type="checkbox"/>	See patent family annex.
* Special categories of cited documents:			
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search		Date of mailing of the international search report	
24 AUGUST 1996		17 SEP 1996	
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231		Authorized officer JEFFREY MULLIS	
Faxsimile No. (703) 305-3230		Telephone No. (703) 308-1235	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08873

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 23 24
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 23 and 24 are drawn to a use which is non-statutory.
2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.